

From: [Ravi Subramaniam](#)  
To: [John Whalan](#)  
Cc: [Sury Vulmiri](#)  
Subject: Re: Fw: Questions about Cap et al. (2008) - Selected ion flow tube mass spectrometry of exhaled breath condensate headspace  
Date: 01/12/2010 08:46 AM  
Signed by: CN=Ravi Subramaniam/OU=DC/O=USEPA/C=US

(b) (5)

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▼ John Whalan---01/12/2010 08:27:04 AM----- Forwarded by John Whalan/DC/USEPA/US on 01/12/2010 08:26 AM -----

From: John Whalan/DC/USEPA/US  
To: Ravi Subramaniam/DC/USEPA/US@EPA, Sury Vul miri/DC/USEPA/US@EPA  
Date: 01/12/2010 08:27 AM  
Subject: Fw: Questions about Cap et al. (2008) - Selected ion flow tube mass spectrometry of exhaled breath condensate headspace

----- Forwarded by John Whalan/DC/USEPA/US on 01/12/2010 08:26 AM -----

From: Petr k Spanel <spane@jh-inst.cas.cz>  
To: John Whalan/DC/USEPA/US@EPA  
Cc: Smith David <d.smith@bemp.keele.ac.uk>, Ksen ya Dryah na <dryahina@yahoo.com>, petr.cap@homolka.cz  
Date: 01/12/2010 06:14 AM  
Subject: Re: Questions about Cap et al. (2008) - Selected ion flow tube mass spectrometry of exhaled breath condensate headspace

Dear Dr Whalan.

Thank you for your interest in our work.

The United States Environmental Protection Agency is preparing a cancer and non-cancer assessment of formaldehyde for the Integrated Risk Information System (IRIS). I am the chemical manager for this project.

An important issue for our assessment is to compare our guideline levels with estimates of formaldehyde in exhaled breath. We have evaluated three studies: Moser et al. (2005), Turner et al. (2008), and Cap et al. (2008), for which you are a co-author.

I am co-author only of the last two, not Moser.

Of these, your study provides the best information because you had a low limit of detection (3 ppb or better) and a low formaldehyde concentration in the laboratory air. I would appreciate it if you could provide me with some additional information regarding your study, your formaldehyde measurements, and your conclusions:

The objective of the Cap et al. study was to compare EBC with direct analysis, formaldehyde was one of many compounds recorded. Below I will give further references to our formaldehyde results.

1. Did any of your volunteers smoke cigarettes? If yes, did this affect the formaldehyde measurements in the direct breath and EBC?

Two of the subjects were smokers, the rest declared to be non-smokers. The two smokers had formaldehyde in the lower range (0-3 ppb).

2. On page 2846, it says that the limit of quantification for all gases measured in your study was 3 ppb or better. Most of the formaldehyde measurements ranged from 0-3 ppb, however. How much confidence should we have in these measurements?

This means that the recorded breath level was below 3 ppb.

3. Your study routinely measured the ambient levels of the gases, but the levels are not provided in the paper. It will be very useful for us to know the ambient values for formaldehyde. Could you please provide these measurements?

Attached is example of raw data from that study.



This plot shows time profile of several concentrations, formaldehydeBAMOD is shown in yellow. A portion of ambient air evaluated numerically is indicated by the white region. Note that ammonia is elevated in three recorded exhalations as is water vapor. However formaldehyde decreases in the exhalations below the ambient level that is 9.6±1.5 ppb. This might be because the subjects were coming to the room directly from outdoors and were not exposed to the indoors air for more than a few minutes.

4. Indoor levels of formaldehyde are typically in the range of 10-40 ppb. The ambient formaldehyde concentration in your study was 3 ppb or less, which is very low for indoor air. Why was it so low? Was the laboratory air filtered through activated charcoal to eliminate the VOCs?

See above, the raw data indicate ambient level of about 10 ppb. This agrees with our results from perhaps more relevant study (pdf attached)

Analysis of breath, exhaled via the mouth and nose, and the air in the oral cavity  
Tianshu Wang, Andriy Pysanenko, Kseniya Dryzhina, Patrik Španěl, David Smith  
Journal of Breath Research 2 (2008) 037013 (13pp)

[doi:10.1088/1752-7155/2/3/037013](https://doi.org/10.1088/1752-7155/2/3/037013)

Note that in table 1 the range of the median exhaled formaldehyde concentrations is 4 to 7 ppb and the indoor air level is 9±3 ppb. Figure 14 shows the distributions, often the exhaled level is lower than ambient but sometimes it is slightly higher. The conclusion was that the origin of formaldehyde in breath is uncertain but the levels agree with study [36] done by independent laboratory on a larger cohort

[36] Wehinger A, Schmid A, Mechtcheriakov S, Ledochowski M, Grabmer C, Gastl G A and Amann A 2007 Lung cancer detection by proton transfer reaction mass-spectrometric analysis of human breath gas Int. J. Mass Spectrom. 265 49-59

5. I am not sure how to interpret the scatter plot for formaldehyde in figure 6. Most volunteers had low formaldehyde concentrations in EBC and direct breath. There were, however, several volunteers with high formaldehyde concentrations in the EBC (6-12 ppb) but low formaldehyde concentrations in direct breath (0-4 ppb). Were these smokers?

No. The two smokers had low formaldehyde concentrations in EBC and direct breath.

Conversely, there were several volunteers with high formaldehyde concentrations in direct breath (4-12 ppb) but very low formaldehyde concentrations in the EBC. I would appreciate your thoughts on interpreting these data.

This is presumably due to the different and uncontrolled exogenous origin, different durations and levels of exposure (some volunteers were staff who worked in unknown environment all day). We were puzzled by the lack of correlation between direct breath and EBC headspace. EBC is collected from total breath during 10 minutes with a noseclip.

Is there an association between formaldehyde measurements and smoking or health status?

Not in this study. It seems to be mostly exogenous. We were hypothesizing that formaldehyde is related to lung cancer, but this was not confirmed by a clinical study so far.

6. Are you aware of any similar studies that have been performed lately for formaldehyde?

See the attached file and the references there in. You might also look at the abstract of four papers in JBR including this one below. Kushch addresses the smokers specifically.

We are in the final stages of writing our assessment, so a rapid reply would be greatly appreciated.

Please excuse brevity of my answer and a lack of polite style, it is not a sign of disrespect but rather I tried to write an immediate answer.

Let us know if you need any more information. Also my colleague Prof. David Smith FRS from Keele might have some further insight into these issues (email in cc [d.smith@bemp.keele.ac.uk](mailto:d.smith@bemp.keele.ac.uk)).

Thank you.

Hope that you find this useful. We would appreciate if you could share with us any further information on the origin of formaldehyde in exhaled breath, as it is still an open question.

With best regards,

Patrik.

**Title:** Quantification of trace levels of the potential cancer biomarkers formaldehyde, acetaldehyde and propanol in breath by SIFT-MS

**Author(s):** Patrik Španel and David Smith

**Affiliation(s):** J Heyrovsky Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Dolejskova 3, 182 23, Prague 8, Czech Republic; Institute for Science and Technology in Medicine, School of Medicine, Keele University, Thornburrow Drive, Hartshill, Stoke-on-Trent, ST4 7QB, UK

**Publication date:** December 2008

**Volume:** 2 **Start page:** 046003

**Publication:** Journal of Breath Research

**URL:** <http://stacks.iop.org/1752-7163/2/046003>

**Abstract:**

The sensitivity of selected ion flow tube mass spectrometry, SIFT-MS, has been increased such that it is now possible to detect metabolites present at a part-per-billion, ppb, level in single breath exhalations. However, to utilize this improved sensitivity, the overlaps (coincidences) of those ions resulting from interfering reactions of impurity precursor ions with some breath metabolites present at higher concentrations with the analytical product ions characteristic of particular metabolites must be accounted for. In this paper, the full reaction schemes are presented for SIFT-MS analyses of three volatile potential cancer biomarkers in exhaled breath, namely formaldehyde, HCHO, acetaldehyde, CH<sub>3</sub>CHO and 2-propanol, CH<sub>3</sub>CH(OH)CH<sub>3</sub>, which identify both the characteristic SIFT-MS product ions for these compounds and the interfering ions at the same mass-to-charge, *m/z*, values. An absolute quantification equation accounting for these interferences is formulated and appropriate entries into the SIFT-MS kinetics library are indicated. **It is shown that when using H<sub>2</sub>O<sup>+</sup> to quantify formaldehyde and acetaldehyde the reactions of impurity O<sub>2</sub><sup>+</sup> ions with methanol and ethanol (always present in breath) must be accounted for** and that the quantification of acetaldehyde must avoid the interference of the CO<sub>2</sub> present in exhaled breath. Finally, it is indicated that the analysis of 2-propanol can be achieved using both H<sub>2</sub>O<sup>+</sup> and NO<sup>+</sup> precursor ions.

**Title:** Analysis of breath, exhaled via the mouth and nose, and the air in the oral cavity

**Author(s):** Tianshu Wang, Andriy Pysanenko, Kseniya Dryahina, Patrik Španel and David Smith

**Affiliation(s):** Institute for Science and Technology in Medicine, School of Medicine, Keele University, Thornburrow Drive, Hartshill, Stoke-on-Trent, ST4 7QB, UK; J Heyrovsky Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Dolejskova 3, 182 23, Prague 8, Czech Republic

**Publication date:** September 2008

**Volume:** 2 **Start page:** 037013

**Publication:** Journal of Breath Research

**URL:** <http://stacks.iop.org/1752-7163/2/037013>

**Abstract:**

Analyses have been performed, using on-line selected ion flow tube mass spectrometry (SIFT-MS), of the breath of three healthy volunteers, as exhaled via the *mouth* and the *nose* and also of the air in the oral *cavity* during breath hold, each morning over a period of one month. Nine trace compounds have been quantified and concentration distributions have been constructed. Of these compounds, the levels of acetone, methanol and isoprene are the same in the *mouth*-exhaled and the *nose*-exhaled breath; hence, we deduce that these compounds are totally systemic. The levels of ammonia, ethanol and hydrogen cyanide are much lower in the *nose*-exhaled breath than in the *mouth*-exhaled breath and highest in the oral *cavity*, indicating that these compounds are largely generated in the mouth with little being released at the alveolar interface. Using the same ideas, both the low levels of propanol and acetaldehyde in *mouth*-exhaled breath appear to have both oral and systemic components. **Formaldehyde is at levels in *mouth*- and *nose*-exhaled breath and the oral *cavity* that are lower than that of the ambient air and so its origin is difficult to ascertain, but it appears to be partially systemic.** These results indicate that serious contamination of alveolar breath exhaled via the *mouth* can occur and if breath analysis is to be used to diagnose metabolic disease then analyses should be carried out of both *mouth*- and *nose*-exhaled breath to identify the major sources of particular trace compounds.

**Title:** Compounds enhanced in a mass spectrometric profile of smokers' exhaled breath versus non-smokers as determined in a pilot study using PTR-MS

**Author(s):** Ievgeniia Kusch, Konrad Schwarz, Lukas Schwentner, Bettina Baumann, Alexander Dzien, Alex Schmid, Karl Unterkofler, Günter Gastl, Patrik Španel, David Smith and Anton Amann

**Affiliation(s):** Department of Anaesthesiology and Critical Care Medicine, Innsbruck Medical University, Anichstrasse 35, A-6020 Innsbruck, Austria; Breath Research Unit of the Austrian Academy of Sciences, Dammstrasse 22, A-6850 Dornbirn, Austria; Burgerstrasse 2, A-6020 Innsbruck, Austria; Fachhochschule Vorarlberg, Hochschulstrasse 1, A-6850 Dornbirn, Austria; Division of Haematology and Oncology, Innsbruck Medical University, Anichstrasse 35, A-6020 Innsbruck, Austria; V Cermak Laboratory, J Heyrovsky Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Dolejskova 3, 18223 Prague 8, Czech Republic; Institute for Science and Technology in Medicine, Medical School, Keele University, Thornburrow Drive, Hartshill, Stoke-on-Trent, ST4 7QB, UK

**Publication date:** June 2008

**Volume:** 2 **Start page:** 026002

**Publication:** Journal of Breath Research

**URL:** <http://stacks.iop.org/1752-7163/2/026002>

**Abstract:**

A pilot study has been carried out to define typical characteristics of the trace gas compounds in exhaled breath of non-smokers and smokers to assist interpretation of breath analysis data from patients who smoke with respiratory diseases and lung cancer. Exhaled breath was analyzed using proton transfer reaction-mass spectrometry (PTR-MS) for 370 volunteers (81 smokers, 210 non-smokers, 79 ex-smokers). Volatile organic compounds corresponding to product ions at seven mass-to-charge ratios (*m/z* 28, 42, 69, 79, 93, 97, 123) in the PTR-MS spectra differentiated between smokers and non-smokers. The Youden index (= maximum of sensitivity + specificity - 1, YI) as a measure for differentiation between smokers and non-smokers was YI = 0.43 for ions at the *m/z* values 28 (tentatively identified as HCN), YI = 0.75 for *m/z* = 42 (tentatively identified as acetonitrile) and YI = 0.53 for *m/z* = 79 (tentatively identified as benzene). **No statistically significant difference between smokers and non-smokers was observed for the product ions at *m/z* = 31 and 33 (compounds tentatively identified as formaldehyde and methanol).** When interpreting the exhaled breath of lung cancer or COPD patients, who often smoke, compounds appearing at the above-mentioned seven mass-to-charge ratios should be considered with appropriate care to avoid misdiagnosis. Validation studies in larger numbers of patients with more precise delineation of their smoking behavior and using additional analytical techniques such as GC/MS and SIFT-MS should be carried out.

**Title:** Recent advances of laser-spectroscopy-based techniques for applications in breath analysis

**Author(s):** Matthew R McCurdy, Yuri Bakhrin, Gerard Wysocki, Rafal Lewicki and Frank K Tittel

**Affiliation(s):** Rice Quantum Institute, Rice University, 6100 Main St., Houston, TX 77005, USA; Medical Scientist Training Program, Baylor College of Medicine, 1 Baylor Plaza, Houston, TX 77030, USA

**Publication date:** September 2007

**Volume:** 1 **Start page:** 014001

**Publication:** Journal of Breath Research

**URL:** <http://stacks.iop.org/1752-7163/1/014001>

**Abstract:**

Laser absorption spectroscopy (LAS) in the mid-infrared region offers a promising new effective technique for the quantitative analysis of trace gases in human breath. LAS enables sensitive, selective detection, quantification and monitoring in real time, of gases present in breath. This review summarizes some of the recent advances in LAS based on semiconductor lasers and optical detection techniques for clinically relevant exhaled gas analysis in breath, specifically such molecular biomarkers as nitric oxide, ammonia, carbon monoxide, ethane, carbonyl sulfide, **formaldehyde** and acetone.[attachment "2008-144.pdf" deleted by Ravi Subramaniam/DC/USEPA/US]